High kynurenine:tryptophan ratio is associated with liver fibrosis in HIV-monoinfected and HIV/hepatitis C virus-coinfected women

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Introduction

- Chronic liver disease is a leading cause of morbidity and mortality in persons living with HIV (PLWH).
- PLWH who are coinfected with hepatitis C virus (HCV) experience accelerated liver fibrosis progression compared to those with HCV alone.
- Alterations in the gut microbiome are thought to play a role in liver disease pathogenesis in PLWH
- Increased gut permeability allows bacterial translocation of lipopolysaccharide (LPS), upregulating proinflammatory and profibrogenic cytokines in the liver
- Tryptophan catabolism has been identified as an important pathway in HIV pathogenesis. Indoleamine 2,3-dioxygenase (IDO-1), an enzyme that catabolizes tryptophan to kynurenine, is activated in HIV and promotes immune dysregulation, leading to depression, cardiovascular disease, and mortality.
- However, its potential role in mediating liver fibrosis has not been examined.
- **The objective of our study was to determine the association of IDO1 activity, measured by the kynurenine:tryptophan ratio (KTR), with liver fibrosis in PLWH, with or without HCV coinfection.**

Methods

Study Population

- Participants of the San Francisco Women's Interagency HIV Study (WIHS) who underwent MR spectroscopy from 12/2003 to 7/2015 were included.
- Subjects were excluded if they had hepatitis B surface antigenemia, prior HCV treatment, history of decompensated cirrhosis, or if plasma samples were not available for KTR testing

Measurements

- Plasma tryptophan and kynurenine levels were measured using plasma samples within six months of their MR spectroscopy in 137 women
- Liquid chromatography-tandem mass spectrometry was used to assess kynurenine and tryptophan levels
- FIB-4 was measured at the same visit as kynurenine and tryptophan levels
- Liver stiffness measurement (LSM) using vibration-controlled transient elastography was obtained in a subset of women (n=83)

Covariates

- Alcohol was categorized as: none; light (>0-7 drinks/week); moderate (7-12 drinks/week); or heavy (>12 drinks/week).
- Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and glucose values.
- Liver fat fraction (LFF) was calculated from the corrected MRS measures of CH_2 and CH_3 lipids and of water as the total lipids / (total lipids + water). The LFF was multiplied by 100 and expressed as a percent.
- Visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) volumes were generated based on magnetic resonance slices located at the discs between lumbar vertebrae L2-3, L3-4, and L4-5.
- Probable cirrhosis was defined as APRI>2 or FIB-4>3.25.

Statistical Analysis

- We compared demographic and clinical characteristics by disease category using an ANOVA model, Kruskal-Wallis, chi-squared test or Fisher's exact test, where appropriate.
- Multivariable linear regression models were used to determine the factors associated with fibrosis (as measured by FIB-4 and LSM) in the total cohort and in the HIV-infected-cohort. We then added KTR to the models to determine the independent association of KTR with fibrosis and to evaluate whether adjustment for KTR altered the associations of HIV and HCV with fibrosis.
- We performed an interaction analysis to determine whether HIV or HIV/HCV modified the association of KTR and fibrosis (or vice versa).
- Markov chain Monte Carlo method with 25 repetitions was used to impute missing covariates.

Results

Table 1. Demographic and clinical characteristics stratified by infection status

Median (IQR) or n (%)	HIV-monoinfected (n=59)	HIV/HCV-coinfected (n=42)	HIV-uninfected (n=36)	p-value
Demographics				
Age	50 (46, 55)	52 (47, 57)	42 (37, 53)	0.002
Race				0.269
African American	28 (48%)	26 (62%)	21 (58%)	
White	14 (24%)	6 (14%)	7 (19%)	
Hispanic	8 (14%)	9 (21%)	5 (14%)	
Other	9 (15%)	1 (2%)	3 (8%)	
Lifestyle				
Alcohol				0.982
None	26 (44%)	21 (53%)	19 (53%)	
0-7 drinks/week	26 (44%)	15 (38%)	13 (36%)	
7-12 drinks/week	4 (7%)	2 (5%)	2 (6%)	
>12 drinks/week	3 (5%)	2 (5%)	2 (6%)	
Current smoker	19 (32%)	28 (67%)	15 (42%)	0.003
Current marijuana user	22 (38%)	10 (25%)	18 (49%)	0.098
IDU ever	5 (9%)	27 (64%)	5 (14%)	<0.001
Metabolic				
BMI (kg/m^2)	27 (23, 33)	24 (22, 32)	32 (29, 38)	0.001
Waist Circumference (cm)	95 (85, 102)	88 (82, 102)	104 (93, 118)	0.002
Steatosis (LFF)	0.01 (0.01, 0.04)	0.02 (0.01, 0.04)	0.02 (0.01, 0.1)	0.080
VAT (cm^3)	145 (104, 187)	120 (85, 197)	169 (99, 212)	0.503
Abdominal SAT (cm^3)	296 (210, 429)	255 (191, 348)	440 (201, 523)	0.067
HOMA-IR	1.87 (1.21, 3.62)	2.89 (1.78, 4.41)	2.22 (1.13, 3.45)	0.292
Liver-related				
AST level, U/L	19 (16, 26)	41 (28, 65)	20 (15, 22)	<0.001
ALT level, U/L	15 (13, 22)	30 (21, 52)	16 (13, 22)	<0.001
Platelet count	250 (213, 290)	192 (158, 254)	278 (230, 359)	<0.001
APRI	0.25 (0.19, 0.3)	0.67 (0.31, 1.04)	0.2 (0.12, 0.25)	<0.001
FIB-4	0.98 (0.79, 1.53)	2.17 (1.24, 3.38)	0.63 (0.57, 0.92)	<0.001
Presence of cirrhosis	2 (3.4%)	11 (26.2%)	0 (0%)	<0.001
HIV-related factors				
HIV RNA (copies/mL)	48 (20, 139)	80 (41, 871)	N/A	0.031
HIV RNA undetectable	35 (59%)	12 (29%)	N/A	0.002
CD4 (/microL)	567 (445, 752)	406 (216, 638)	N/A	0.005
CD4 nadir	229 (100, 288)	148 (108, 236)	N/A	<0.001
% currently on HAART	49 (83%)	33 (79%)	N/A	0.57
History of clinical AIDS	21 (36%)	26 (62%)	N/A	0.01

Abbreviations: AIDS=acquired immunodeficiency syndrome, APRI=AST to platelet ratio index, AST=aspartate aminotransferase, ALT=alanine aminotransferase, FIB-4=fibrosis-4 score, HAART=highly active antiretroviral therapy, HCV=hepatitis C virus, HIV=human immunodeficiency virus, HOMA-IR=Homeostatic Model Assessment of Insulin Resistance, IDU=injection drug use, LFF=liver fat fraction, RNA=ribonucleic acid, SAT=subcutaneous adipose tissue, U/L=units per liter, VAT=visceral adipose tissue

Figure 1. Median KTR by infection status. KTR was highest in the HIV/HCV-coinfected group (0.056 [IQR: 0.045-0.066]), followed by HIV-monoinfected women (0.038 [IQR: 0.032-0.046]), and was lowest (0.031 [IQR: 0.025-0.034]), in the uninfected women (p<0.001)

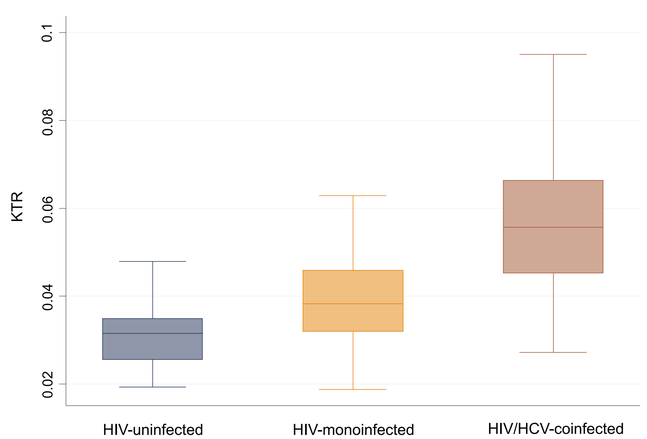


Table 2. HIV monoinfection and HIV/HCV coinfection are associated with higher FIB-4 in the total cohort (n=137), independent of KTR, demographic, lifestyle, and metabolic factors

	Adjusted model*		Adjusted model with KTR	
Variables	**% effect (95% CI)**	**p-value**	**% effect (95% CI)**	**p-value**
HIV monoinfection	37% (9%, 73%)	0.008	29% (2%, 63%)	0.035
HIV/HCV coinfection	164% (100%, 250%)	<0.001	123% (63%, 203%)	<0.001
KTR	N/A	N/A	27% (5%, 53%)	0.013

* Multivariable model also adjusted for age, race, alcohol, marijuana, history of injection drug use, steatosis, visceral adipose tissue, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance)

Table 3. HIV/HCV coinfection, lower CD4 count, and higher KTR are associated with FIB-4 in HIV-infected persons (n=101) in multivariable analysis*

	Adjusted model*		Adjusted model with KTR	
Variables	**% effect (95% CI)**	**p-value**	**% effect (95% CI)**	**p-value**
HIV/HCV coinfection	68% (26%, 124%)	<0.001	52% (14%, 104%)	0.005
CD4 count	-5.6 (-9.8%, -1.1%)	0.016	-4.5% (-8.8%, 0%)	0.050
KTR	N/A	N/A	29% (4%, 59%)	0.020

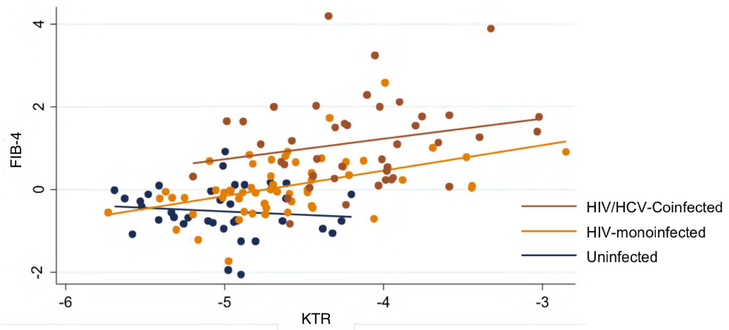
* Multivariable model also adjusted for age, race, alcohol, marijuana, history of injection drug use, steatosis, visceral adipose tissue, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance)

Table 4. HIV/HCV coinfection and higher KTR are associated with greater liver stiffness measurement in the total cohort (n=83) in multivariable analysis*

	Adjusted model		Adjusted model with KTR	
Variables	% effect (95% CI)	p value	% effect (95% CI)	p value
HIV monoinfection	5% (-19%, 37%)	0.711	-1% (-23%, 27%)	0.941
HIV/HCV coinfection	94% (40%, 170%)	<0.001	47% (3%, 110%)	0.034
KTR	N/A	N/A	43% (15%, 79%)	0.002

* Multivariable model also adjusted for age, race, alcohol, marijuana, history of injection drug use, steatosis, visceral adipose tissue, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance)

Figure 2. Higher KTR is associated with greater FIB-4 in HIV/HCV-coinfected and HIV-monoinfected, but not HIV-uninfected women*



*Higher KTR was associated with greater FIB-4 in the HIV/HCV-coinfected cohort (r= 0.17, p=0.17) and HIV-monoinfected (Spearman r= 0.51, p<0.001) women, but not in the uninfected women (r= -0.11, p=0.51)

Conclusions

- Higher KTR is associated with greater liver fibrosis, as measured by both FIB-4 and LSM
- KTR is higher in HIV-monoinfected and HIV/HCV-coinfected women
- The association between HIV infection and fibrosis was attenuated after including KTR in multivariable models, suggesting that KTR may partly explain the association
- These findings are among the first to provide evidence that tryptophan catabolism may be clinically relevant in liver fibrosis progression in the setting of HIV
- Whether this pathway is causally associated with fibrosis requires further investigation

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